FORM PTO-1390 (REV 11-98) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER: **ENDO=12**

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known see 37 CFR 1.5): 09/485583

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP98/03581	**12 August 1998**	**12 August 1997**

TITLE OF INVENTION: **REMEDIES FOR DISEASES ASSOCIATED WITH BONE RESORPTION**

APPLICANT(S) FOR DO/EO/US: **Koichi ENDO et al.**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).
4. ☒ A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. ☒ A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   a. ☐ is transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☒ has been transmitted by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☒ A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   a. ☐ are transmitted herewith (required only if not transmitted by the International Bureau).
   b. ☐ have been transmitted by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
    ☒ A SECOND or SUBSEQUENT preliminary amendment.
14. ☐ A substitute specification.
15. ☐ A change of power of attorney and/or address letter.
16. ☒ Other items or information:
    1. **A courtesy copy of the first page of the International Publication (WO99/07412).**
    2. **A courtesy copy of the International Search Report.**
    3. **A courtesy copy of the International Preliminary Examination Report.**
    4. **Formal drawings, 1 sheet, figure 1.**

U.S. APPLICATION NO. (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
09/485583	PCT/JP98/03581	ENDO=12

	CALCULATIONS PTO USE ONLY
17. ☒ The following fees are submitted:	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :	
Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $970.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $840.00	
International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO $760.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) but all claims did not satisfy provisions of PCT Article 33(1)-(4) $670.00	
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(1)-(4) $96.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ **840.00**
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE	
Total claims	**14** - 20 =	**0**	X $18.00	$ **0**
Independent claims	**2** - 3 =	**0**	X $78.00	$ **0**
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $260.00	$

TOTAL OF ABOVE CALCULATIONS =	$ **840.00**
Reduction of 1/2 for filing by small entity, if applicable. A Small Entity Statement must also by filed (Note 37 CFR 1.9, 1.27, 1.28).	$
SUBTOTAL =	$ **840.00**
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +	$
TOTAL NATIONAL FEE =	$ **840.00**
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00** per property +	$
TOTAL FEES ENCLOSED =	$ **840.00**
Amount to be: refunded	$
charged	$

a. ☒ A check in the amount of $ **840.00** to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. _____ in the amount of $_____ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **02-4035**. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pendin g status.

SEND ALL CORRESPONDENCE TO

BROWDY AND NEIMARK, P.L.L.C.
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SIGNATURE

Roger L. Browdy
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25,618
REGISTRATION NUMBER

Date of this submission: February 14, 2000

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:	Art Unit:
Koichi ENDO et al.	
IA No.: PCT/JP98/03581	Washington, D.C.
IA Filed: August 12, 1998	
U.S. App. No.: (Not Yet Assigned)	February 14, 2000
National Filing Date: (Not Yet Received)	
For: REMEDIES FOR DISEASES …	Docket No.: ENDO=12

PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Contemporaneous with the filing of this case and prior to calculation of the filing fee, kindly amend as follows:

IN THE SPECIFICATION

After the title please insert the following paragraph:

--CROSS REFERENCE TO RELATED APPLICATION

The present application is the national stage under 35 U.S.C. 371 of PCT/JP98/03581, filed August 12, 1998. --

REMARKS

The above amendment to the specification is being

made to insert reference to the PCT application of which the present case is a U.S. national stage.

Favorable consideration and allowance are earnestly solicited.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
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In re Application of:	Art Unit:
Koichi ENDO et al.	
IA No.: PCT/JP98/03581	Washington, D.C.
IA Filed: August 12, 1998	
U.S. App. No.: (Not Yet Assigned)	February 14, 2000
National Filing Date: (Not Yet Received)	
For: REMEDIES FOR DISEASES ...	Docket No.: ENDO=12

SUPPLEMENTAL PRELIMINARY AMENDMENT

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Prior to examination upon the merits, kindly amend as follows:

IN THE CLAIMS

Please cancel claims 1-7 without prejudice in favor of the following new claims 8-21:

--8. A method for treating a bone resorption-associated disease comprising administering to a subject in need thereof an effective amount of a selective iNOS inhibitor.

--9. The method as claimed in claim 8, wherein the bone resorption-associated disease is osteoporosis.

--10. The method as claimed in claim 8, wherein the selective iNOS inhibitor is used as a bone mass-maintenance drug.

--11. The method as claimed in claim 8, wherein the selective iNOS inhibitor is used as a bone resorption retardant.

--12. The method as claimed in claim 8, wherein the selective iNOS inhibitor is used as an inhibitor of bone metastasis of tumor cells.

--13. The method as claimed in claim 8, wherein the bone resorption-associated disease is nephritis.

--14. The method as claimed in claim 8, wherein the selective iNOS inhibitor is used as a progression retardant of chronic renal failure.

--15. A kit for treating a bone resorption-associated disease comprising an effective amount of a selective iNOS inhibitor and instructions for treating a bone resorption-associated disease.

--16. The kit as claimed in claim 15, wherein the bone resorption-associated disease is osteoporosis.

--17. The kit as claimed in claim 15, wherein the selective iNOS inhibitor is used as a bone mass-maintenance drug.

--18. The kit as claimed in claim 15, wherein the selective iNOS inhibitor is used as a bone resorption retardant.

--19. The kit as claimed in claim 15, wherein the selective iNOS inhibitor is used as an inhibitor of bone metastasis of tumor cells.

--20. The kit as claimed in claim 15, wherein the bone resorption-associated disease is nephritis.

--21. The kit as claimed in claim 15, wherein the selective iNOS inhibitor is used as a progression retardant of chronic renal failure.--

REMARKS

Claims 8-21 presently appear in this case. The above amendments to the claims are being made in order to place the application in better condition for examination.

Favorable consideration is earnestly solicited.

Respectfully submitted,

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1/PRT

SPECIFICATION

THERAPEUTICS OF BONE RESORPTION-ASSOCIATED DISEASES

TECHNICAL FIELD

This invention relates to drugs for treating bone resorption-associated diseases in the occurrence or development of which iNOS participates. Namely, the present invention relates to therapeutics of bone resorption-associated diseases containing selective iNOS inhibitors as the active ingredient. More particularly, it relates to therapeutics of bone resorption-associated diseases containing selective iNOS inhibitors as the active ingredient which are to be used as a therapeutic of osteoporosis, a bone mass-maintenance drug, a bone resorption retardant, an inhibitor of bone metastasis of tumor cells, a therapeutic of nephritis, a progression retardant of chronic renal failure, etc.

BACKGROUND ART

In recent years, it has been reported that nitric oxide (hereinafter referred to simply as NO), which has been considered as endothelium-derived relaxing factor, exerts various effects in a number of tissues (Nathan, C.F. & Hibbs, J.B.Jr., Curr. Opin. Immunol., 3:65-70, 1990, Liew, F.Y. & Cox, F.E.G., Immunol. Today, 12:A17-21, 1991). NO production is controlled by NO synthase (NOS) and it is known at present that NOS exists in three isoforms (Forstermann, U., Schmidt, H.H.H.W., et al., Biochem. Pharmacol. 42:1849-1857, 1991). It is pointed out that,

among these isoforms, type II NOS (inducible NOS: iNOS) might participate in various diseases, since its expression is controlled by various cytokines (Moncada, S., et al., Pharmacol. Rev., 43:109-142, 1991, Nathan, C., FASEB J., 6:3051. 1992).

Recently, NO has become the center of attention as a bone metabolism regulator. It has been reported that nitroglycerin, which is an NO donor, counteracts the bone loss associated with ovariectomy (Wimalawansa S.J., et al., Bone 18:301-304, 1996). It has been also reported that pit formation serving as an indication of bone resorption is reduced by sodium nitropusside (SNP) which is another NO donor (Kasten T.P., et al., Proc. Natl. Acad. Sci. USA, 91:3569-3573, 1994). Based on these reports, it has been considered that NO would have therapeutic effect on osteoporosis (Schmidt, H.H.H.W. et al., J. Histochem. Cytochem. 40:1439-1456, 1992). On the other hand, it is known that inflammatory cytokines participating in osteoporosis (IL-1, TNF-α, etc.) enhance iNOS and thus accelerate NO production (Mika Hukkanen, et al., Endocrinology, 136;5445-5453, 1995).

Recently, Chow J.W.M., et al. reported in the American Society for Bone and Mineral Research that not iNOS but type I NOS (neural-constitutive NOS) and type III NOS (endothelial-constitutive NOS) are exclusively expressed in normal human bone tissues (Bone Miner. Res., 11, supplement 1:M354, 1996).

On the other hand, it is known that active bone

resorption is observed in the attachment area of cancer cells upon bone tissues (Eilon G., Mundy GR., Nature, 276:726-728, 1978, Mundg GR. Raisz LG, et al., N. Engl. J. Med., 291:1041-1046, 1974).

Moreover, it has been known that the first signal of the induction of nephritis is the activation of NF-kB gene followed by the activation of iNOS gene (Xie, et al., J. Exp. Med., 177:1779-1784, 1994).

As described above, NO relates to various bone resorption-associated diseases typified by osteoporosis as well as bone metastasis of tumor cells, nephritis and chronic renal failure.

WO (International patent application opened public) 96-30350 discloses amidine derivatives which are useful as therapeutics of diseases in which NOS participates and osteoporosis is cited therein as an example of these diseases. However, nothing but an inhibitory activity on nNOS is disclosed in this patent as concrete specific data of these compounds. .

As stated above, iNOS participates closely in bone metabolism and relates to bone resorption.

The therapeutics according to the present invention are efficacious entirely against bone resorption-associated diseases, in particular, osteoporosis, bone metastasis of tumor cells, nephritis, chronic renal failure, etc.

With the coming of the aging society, osteoporosis has attracted public attention not only as a medical problem but also as a serious social problem. Although it has been

a practice to treat osteoporosis with the use of estrogen, calcitonin, active form of vitamin D, vitamin K, bisphosphonate, etc., these drugs are accompanied respectively by the problems of rejuvenation, drug resistance, hypercalcemia, hemolysis, drug resistance, etc. Thus, none of these drugs can establish a sufficient therapeutic effect from a clinical viewpoint.

On the basis of the relation between the attachment take of cancer cells upon bone tissues and bone resorption as described above, it is expected that the bone metastasis of tumor cells can be inhibited by controlling bone resorption. Accordingly, it can be said that bone metastasis of tumor cells also falls within the category of the bone resorption-associated diseases.

Since nephritis is induced by the activation of iNOS gene following the activation of NF-kB gene, it is highly meaningful in treating nephritis to selectively inhibit iNOS. Furthermore, the selective inhibition of iNOS is also meaningful in ameliorating uremic symptom in chronic renal failure and retarding the introduction of dialysis.

DISCLOSURE OF THE INVENTION

The present invention, which relates to drugs for treating diseases in the occurrence or development of which iNOS participates, aims at providing therapeutics of bone resorption-associated diseases containing selective iNOS inhibitors as the active ingredient.

As the results of intensive studies on the assumption that selective inhibition of iNOS would contribute to the

treatment of bone resorption-associated diseases, the present inventors have successfully found that selective iNOS inhibitors are useful in treating bone resorption-associated diseases, thus completing the present invention. Based on the above-mentioned finding on the relation of osteoporosis and NO and NOS, the present inventors assumed that iNOS would be expressed not in the ordinary state but in pathological states and, therefore, osteoporosis could be treated by selectively inhibiting iNOS. As the results of studies from this standpoint, they have found that selective iNOS inhibitors inhibit bone resorption observed in osteoporosis induced by IL-1, TNF-α, etc. and thus relieve decrease in bone mass.

Accordingly, the present invention provides therapeutics for bone resorption-associated diseases which contain as the active ingredient selective iNOS inhibitors.

The present invention also provides the above-described therapeutics of bone resorption-associated diseases which are to be used as a therapeutic of osteoporosis, a bone mass-maintenance drug, a bone resorption retardant, an inhibitor of bone metastasis of tumor cells, a therapeutic of nephritis, a progression retardant of chronic renal failure, etc.

BRIEF DESCRIPTION OF DRAWING

Fig. 1 is a graph which shows the inhibitory effect of the therapeutic according to the present invention on decrease in lumbar bone density.

BEST MODE FOR CARRYING OUT THE INVENTION

The term "selective iNOS inhibitor" as used herein involves compounds showing extremely weak effect on two constitutive NOS isoforms (i.e., eNOS and nNOS), among the three NOS isoforms, but a selective inhibitory effect on the inducible one (i.e., iNOS). These compounds are not particularly restricted by difference in selectivity to eNOS and nNOS, so long as the inhibitory effect on iNOS exceeds those on eNOS and nNOS. More particularly speaking, it is preferable to use, for example, compounds satisfying any or all of the following requirements. When IC_{50} levels on eNOS, nNOS or cNOS and iNOS are measured by the NOS-inhibitory activity determination method described in Proc. Natl. Acad. Sci. U.S.A. 88:365-369 (1991), the ratio eNOS/iNOS is 25 or above, nNOS/iNOS is 15 or above, or cNOS/iNOS is 15 or above. These compounds involve, for example, low-molecular weight synthetic compounds, peptide compounds and microbial products having the above-described effect. Examples thereof include isothiourea derivatives such as S-alkylisothiourea derivatives and cyclic isothiourea derivatives, amidine derivatives such as chain amidine derivatives and cyclic amidine derivatives, 2-aminopyridine derivatives and guanidine derivatives. Now, particular examples thereof will be cited.

Examples of S-alkylisothiourea derivatives are as follows:

S-ethylisothiourea (EIT) (Can. J. Physiol. Pharmacol., Vol. 73, p. 665, 1995);

S,S'-(1,3-phenylenebis(1,2-ethanedinyl))bis-

isothiourea;

S,S'-(1,4-phenylenebis(1,2-ethanedinyl))bis-isothiourea;

S,S'-((2,5-dimethyl)-(1,4-phenylenebis(1,2-ethanedinyl))bis-isothiourea;

S-(3-methoxyphenethyl)isothiourea;

S-(3-(4-amidinothiomethyl)phenylmethyl)-propyl)isothiourea;

S,S'-(1,4-phenylenebis(1,3-propanedinyl))-bis-isothiourea (The Journal of Biological Chemistry, Vol. 269, No. 43, p. 26669, 1994); etc.

Examples of cyclic isothiourea derivatives are as follows:

3-amino-2-thia-4-aza-cis-bicyclo(4,4,0)-deca-3-ene hydrochloride;

2-amino-trans-5,6-dimethyl-5,6-dihydro-4H-1,3-thiazine hydrobromide;

3-amino-2-thia-4-aza-cis-bicyclo(4,4,0)-nona-3-ene methanesulfonate;

2-amino-trans-4,5-diemthyl-5,6-dihydro-4H-1,3-thiazine;

1(S)-6(R)-4-amino-3-thia-5-aza-cis-bycyclo(4,4,0)-deca-4-ene hydrochloride;

2-amino-cis-5,6-dimethyl-5,6-dihydro-4H-1,3-thiazine methanesulfonate;

S-((2-amino-thiazolyno)methyl)isothiourea;

2-amino-4-hydroxymethyl-thiazoline (WO96/14842);

2-amino-5,6-dihydro-6-methyl-4H-1,3-thiazine (AMT)

(Can. J. Phusiol. Pharmacol., Vol. 73, p. 665, 1995); etc.

Examples of chain amidine derivatives are as follows:

L-N-6-(1-iminoethyl)lysine hydrochloride (NIL);

N-(5S-amino-6,7-dihydroxyheptyl)ethaneimidamide dihydrochloride;

N-(5S-amino-6,7-dihydroxy-6-methylheptyl)ethaneimidamide dihydrochloride dihydrate;

N-(5S-amino-6,7-dihydroxyoctyl)ethaneimidamide dihydrochloride hydrate;

3S-amino-7-((1-iminoetyl)amino)heptanoic acid (WO95/24382);

2-amino-6-(1-iminoethylamino)-4,4'-dioxo-4-thiahexanoic acid;

2-amino-6-(1-imino-2-fluoroethylamino)-4,4-dioxo-4-thiahexanoic acid dihydrobromide;

2-amino-6-(1-iminoethylamino)-4-oxo-4-thiahexanoic acid (WO95/34534); etc.

Examples of cyclic amidine derivatives are as follows:

7-[4,5-dihydro-3-phenylisoxazolyl-5-yl]methyl]-hexahydro-2H-azepin-2-imine monotrifluoroacetate;

(-)-hexahydro-7-(phenylmethyl)-2H-azepin-2-imine monohydrochloride;

(±)(trans)4-methyl-5-(phenylmethyl)pyrrolidin-2-imine monohydrochloride;

hexahydro-7-(phenylmethyl)-2H-azepin-2-imine monohydrochloride;

6-(cyclohexylmethyl)piperidin-2-imine monohydrochloride;

7-(cyclohexylmethyl)hexahydro-2H-azepin-2-imine monohydrochloride;

hexahydro-7-(3-phenylpropyl)-2H-azepin-2-imine monohydrochloride;

hexahydro-7-[(oxiran-2-yl)methyl]-2H-azepin-2-imine monohydrochloride;

hexahydro-7-(3-phenyl-2-propenyl)-2H-azepin-2-imine monohydrochloride (WO96/33175);

2-imino-5(S)-hydroxy-4(S)-methyl-piperidine hydrochloride;

4(S)-methyl-4a(S),8a(S)-decahydro-2-iminoquinoline hydrochloride;

4(R)-methyl-4a(R),8a(R)-decahydro-2-iminoquinoline hydrochloride;

4(S)-methyl-4a(S),7a(S)-perhydro-2-imino-1-pyridine hydrochloride;

4(R)-methyl-4a(R),7a(R)-perhydro-2-imino-1-pyridine hydrochloride;

5(R)-methyl-2-imino-piperidine hydrochloride;

4(R),5(R)-dimethyl-2-imino-piperidine hydrochloride;

2-imino-5(S)-methoxy-4(S)-methyl-piperidine hydrochloride;

4(R),5(S)-dimethyl-2-imino-piperidine hydrochloride;

trans-decahydro-2-iminoquinoline hydrochloride (WO96/14844); etc.

Examples of 2-aminopyridine derivatives are as follows:

2-amino-4,6-dimethyl-3-nitropyridine;

2-amino-6-benzylpyridine (WO96/02637);

2-amino-6-(2-aminoethyl)-4-methylpyridine (WO96/18616); etc.

The therapeutics of the present invention can be used in the form of various medicinal compositions prepared by blending the selective iNOS inhibitor, i.e., the active ingredient, with physiologically nontoxic solid or liquid pharmaceutical carriers. These medicinal compositions may be used in various dosage forms appropriate for administration methods. Examples of the dosage forms include tablets, granules, pills, capsules, solutions, syrups, suspensions, emulsions, ointments and patches. As the pharmaceutical carriers, use can be made of those commonly employed in the art, for example, fillers, binders, disintegrating agents, lubricating agents, coatings, solubilizing agents, emulsifiers, suspending agents, stabilizers and solvents. The therapeutics according to the present invention can be systemically administered as oral preparations or injections. Alternatively, they may be topically administered as external preparations, etc.

In the present invention, the dose of the selective iNOS inhibitor varies depending on the age and sex of the patient, the severity of the symptom, the administration route, etc. In general, it may be administered to an adult in a dose of 0.01 to 1,000 mg/day, preferably 0.1 to 100 mg/day.

The preventive effect of the inhibitor of bone metastasis of tumor cells according to the present

invention can be confirmed by using a bone metastasis model prepared with the use of Hara cells originating in human pulmonary cancer which undergo bone metastasis at a high frequency.

The effects of the therapeutic of nephritis according to the present invention of retarding the development of nephritis and inhibiting the progression of chronic renal failure can be confirmed by using 5/6 nephrectomized rats.

EXAMPLE

The present invention will be described in greater detail by reference to the following Example, but it should be understood that the invention is not construed as being limited thereto.

EXAMPLE 1: Effect on ovariectomized rats

Female Wistar-Imamichi rats were subjected to ovariectomy (OVX). As test drugs, use was made of L-N-6-(1-iminoethyl)lysine hydrochloride (NIL) and S-ethylisothiourea (EIT) which are known as selective iNOS inhibitors. With respect to NOS inhibitory activity expressed in the ratio of IC_{50}, it is reported that NIL shows cNOS/iNOS of 28 while EIT shows nNOS/iNOS of 19.23 and eNOS/iNOS of 28.46 (Moore, W.M., et al., J. Med. Chem., 37:3886, 1994, Ross Tracey W., et al., Can. J. Pharmacol., 73:665-669, 1995). To confirm the validity of the test system, 17-β-estradiol (βE2) was also employed.

One day after OVX, the rats were divided into 7 groups each having 7 animals. The administration of the drug was started on the day 4 after OVX. As a control, a sham group

(7 animals) was also employed. NIL was orally administered in a dose of 0.1 or 0.02 mg/kg (0.1 ml per 100 g of body weight) 5 times per week for 10 weeks.

Fig. 1 shows the results.

In this graph, the data are expressed in "mean ± standard deviation". According to Dunnet's multiple comparison method, the statistically significant differences from the control ovariectomy group are expressed as follows:

**: $p < 0.01$, ***: $p < 0.001$.

As Fig. 1 shows, the lumber bone density of the control ovariectomy group 24 hours after the final administration was significantly decreased to 85.2% ($p < 0.001$), when the lumber bone density of the sham group was referred to as 100%. In the EIT administration group, on the other hand, the decrease in the bone density was significantly inhibited, namely, 92.6% ($p < 0.01$).

In the 0.1 mg/kg NIL administration group, the decrease in the bone density was somewhat inhibited, i.e., 90.2%. In the 0.02 mg/kg NIL administration group, the bone density (86.8%) was almost comparable to that of the control OVX group.

Table 1 summarizes the biochemical test data of urine collected after the final administration.

TABLE 1

	Biochemical parameter of urine	
	Dose (mg/kg)	D-pyr/Cre
Sham group	solvent	7.66 ± 0.05**
Control OVX group	solvent	11.83 ± 2.02
OVX group with NIL administration	0.1	10.43 ± 1.15
	0.02	8.70 ± 1.59**
OVX group with EIT administration	0.1	9.95 ± 1.71
OVX group with βE2 administration	0.02	4.16 ± 1.45**

According to Dunnet's multiple comparison method, the statistically significant differences from the control ovariectomy group are expressed as follows: **: $p < 0.01$, ***: $p < 0.001$.

Number of animals/group = 7. Mean ± standard deviation.

As Table 1 shows, the excretion of deoxypyridinoline (D-pyr) employed as a bone resorption marker was significantly accelerated ($p < 0.01$) in the control OVX group, compared with the sham group. In the 0.02 mg/kg NIL administration group, the acceleration was significantly inhibited ($p < 0.01$). In the EIT administration group, a tendency toward decrease was observed though no statistically significant difference was found.

INDUSTRIAL APPLICABILITY

The present invention provides therapeutics of bone resorption-associated diseases containing selective iNOS inhibitors as the active ingredient.

CLAIMS

1. A therapeutic of bone resorption-associated diseases which contains a selective iNOS inhibitor as the active ingredient.

2. A therapeutic of bone resorption-associated diseases as claimed in Claim 1 which is to be used as a therapeutic of osteoporosis.

3. A therapeutic of bone resorption-associated diseases as claimed in Claim 1 which is to be used as a bone mass-maintenance drug.

4. A therapeutic of bone resorption-associated diseases as claimed in Claim 1 which is to be used as a bone resorption retardant.

5. A therapeutic of bone resorption-associated diseases as claimed in Claim 1 which is to be used as an inhibitor of bone metastasis of tumor cells.

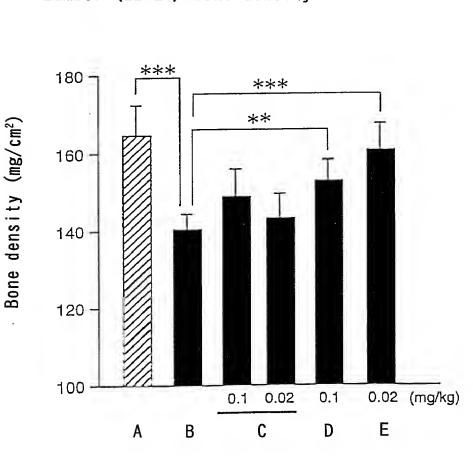
6. A therapeutic of bone resorption-associated diseases as claimed in Claim 1 which is to be used as a therapeutic of nephritis.

7. A therapeutic of bone resorption-associated diseases as claimed in Claim 1 which is to be used as a progression retardant of chronic renal failure.

ABSTRACT

Therapeutics of bone resorption-associated diseases, which contain selective iNOS inhibitors as the active ingredient, are useful as a therapeutic of osteoporosis, a bone mass-maintenance drug, a bone resorption retardant, an inhibitor of bone metastasis of cancer cells, a therapeutic of nephritis, a progression retardant of chronic renal failure, etc.

Fig. 1



A Sham group

B Control ovariectomy group

C Control ovariectomy group with NIL administration

D Control ovariectomy group with EIT administration

E Control ovariectomy group with βE_2 administration

 [X] Original [ ] Supplemental Atty.Docket:

Combined Declaration for Patent Application and Power of Attorney

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name; and that I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

THERAPEUTICS OF BONE RESORPTION-ASSOCIATED DISEASES

the specification of which (check one)

[ ] is attached hereto;

[ ] was filed in the United States under 35 U.S.C. §111 on _____, as USSN _____*; or

[x] was/will be filed in the U.S. under 35 U.S.C. §371 by entry into the U.S. national stage of an international (PCT) application, PCT/JP98/03581; filed August 12, 1998 entry requested on _____*; national stage application received USSN _____*; §371/§102(e) date _____* (*if known), and was amended on _____ (if applicable).

(include dates of amendments under PCT Art. 19 and 34 if PCT)

I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above; and I acknowledge the duty to disclose to the Patent and Trademark Office (PTO) all information known by me to be material to patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. §§ 119, 365 of any prior foreign application(s) for patent or inventor's certificate, or prior PCT application(s) designating a country other than the U.S., listed below with the "Yes" box checked and have also identified below any such application having a filing date before that of the application on which priority is claimed:

(Number)	(Country)	(Day Month Year Filed)	[X] YES	[ ] NO
(Number)	(Country)	(Day Month Year Filed)	[ ] YES	[ ] NO
(Number)	(Country)	(Day Month Year Filed)	[ ] YES	[ ] NO

251264/1997 Japan 12/8/1997

I hereby claim the benefit under 35 U.S.C. § 120 of any prior U.S. non-provisional Application(s) or prior PCT Application(s) designating the U.S. listed below, or under § 119(e) of any prior U.S. provisional applications listed below, and, insofar as the subject matter of each of the claims of this application is not disclosed in such U.S. or PCT application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose to the PTO all information as defined in 37 C.F.R. §1.56(a) which occurred between the filing date of the prior application and the national filing date of this application:

(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)
(Application Serial No.)	(Day Month Year Filed)	(Status: patented, pending, abandoned)

I hereby appoint the following attorneys, with full power of substitution, association, and revocation, to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith.

SHERIDAN NEIMARK, REG. NO. 20,520 - ROGER L. BROWDY, REG. NO. 25,618 - ANNE M. KORNBAU, REG. NO. 25,884
NORMAN J. LATKER, REG. NO. 19,963 - IVER P. COOPER, REG. NO. 28,005 - ALLEN C. YUN, REG. NO. 37,971*
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ADDRESS ALL CORRESPONDENCE TO	DIRECT ALL TELEPHONE CALLS TO:
BROWDY AND NEIMARK, P.L.L.C. 419 Seventh Street, N.W. Washington, D.C. 20004	BROWDY AND NEIMARK (202) 628-5197

The undersigned hereby authorizes the U.S. Attorneys or Agents named herein to accept and follow instructions from YUASA and HARA as to any action to be taken in the U.S. Patent and Trademark Office regarding this application without direct communication between the U.S. Attorney or Agent and the undersigned. In the event of a change of the persons from whom instructions may be taken, the U.S. Attorneys or Agents named herein will be so notified by the undersigned.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

ATTY.'S DOCKET: ENDO=12

In re Application of:	Art Unit:
Koichi ENDO et al.	
	Examiner: ...
Entered: even date herewith	
	Washington, D.C.
I.A. Appln. No.: PCT/JP98/03581	
	February 14, 2000
For: REMEDIES FOR DISEASES…	

NOTICE OF CHANGE OF CORRESPONDENCE ADDRESS

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

Please associate this application with customer number **001444**. Our customer number records show that the new address of Browdy and Neimark, P.L.L.C. is:

BROWDY AND NEIMARK, P.L.L.C.
624 Ninth Street, N.W.
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Our telephone numbers and facsimile numbers remain unchanged.

Respectfully submitted,

BROWDY AND NEIMARK, P.L.L.C.
Attorneys for Applicant(s)

By: _____
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f:\filing\change.frm

Page 2 of 2 Atty.Docket:
Title:
U.S. Application filed _____, Serial No. _____
PCT Application filed _____, Serial No. _____

I hereby further declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. §1001 and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

FULL NAME OF FIRST INVENTOR	INVENTOR'S SIGNATURE	DATE
Koichi ENDO	Koichi Endo	27/8/1999
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FULL NAME OF THIRD JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF FOURTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF FIFTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SIXTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		
FULL NAME OF SEVENTH JOINT INVENTOR	INVENTOR'S SIGNATURE	DATE
RESIDENCE	CITIZENSHIP	
POST OFFICE ADDRESS		

ALL INVENTORS MUST REVIEW APPLICATION AND DECLARATION BEFORE SIGNING. ALL ALTERATIONS MUST BE INITIALED AND DATED BY ALL INVENTORS PRIOR TO EXECUTION. NO ALTERATIONS CAN BE MADE AFTER THE DECLARATION IS SIGNED. ALL PAGES OF DECLARATION MUST BE SEEN BY ALL INVENTORS.